POLYENE MACROLIDE ANTIBIOTICS

The Structures of Fungichromin, Pimaricin and Filipin

By

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POLYENE MACROLIDE ANTIBIOTICS: THE STRUCTURES OF FUNGICHROMIN, PIMARICIN AND FILIPIN*

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The discovery of penicillin and streptomycin and their effect on bacterial infections started the extensive search for microbial metabolites possessing antibiotic properties. A large number of compounds, which are active against a variety of microorganisms, has resulted from these systematic studies. The chemical investigations of the isolated antibiotics have revealed types of structures not earlier encountered in nature. A review discussing the physical, chemical and physiological properties of metabolic products from bacteria, molds, fungi and lichens has recently been published.[1]

The metabolites produced by actinomycetes of the genus *Streptomyces* have been subjected to a more thorough screening for activity against pathogens than probably any similar group. This was a consequence of the antibiotic properties early discovered in compounds produced by species belonging to this genus. Since the majority of these metabolites have been discovered and isolated because of some conspicuous properties, e.g., biological activity, color or insolubility, it would be premature to draw any definite conclusions about their metabolic significance.

The macrolides[2] compose a structurally well defined group of compounds elaborated by *Streptomyces* strains, and they are characterized by a medium or large lactone ring. At the present time, members containing 11—27 carbon atoms in the macrocyclic moiety are known. They display a complicated oxygenation pattern and have mostly a sugar residue of unusual type linked to the macrocyclic nucleus. Macrolides containing a conjugated polyene system form a characteristic subgroup. A large number of tetraenes, pentaenes, hexaenes and heptaenes have been isolated during a systematic biological screening of soil samples collected in different parts of the world. The polyene macrolide antibiotics all display activity against fungi, and some of them have found clinical application.[3] Unfortunately the therapeutic value of these compounds is limited by their toxicity and low solubility in common solvents. At least three reviews on macrolides, also thoroughly discussing the polyene subgroup, have been published.[4, 5, 6]

Fungichromin and filipin, two sugar-free pentaenes, and pimaricin, a tetraene containing the aminohexose mycosamine, are three members of this group. Work describing the structural determination of these macrolides will be surveyed in this paper. The investigations to be discussed have been published elsewhere and will be referred to by the Roman numerals given in the following list.

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* Inaugural dissertation

I. Cope, A. C., Bly, R. K., Burrows, E. P., Ceder, O. J., Ciganek, E., Gillis, B. T., Porter, R. F. and Johnson, H. E., Fungichromin: Complete structure and absolute configuration at C-26 and C-27. *J. Am. Chem. Soc.* **84,** 2170 (1962).
II. Ceder, O., Pimaricin 1: Determination of the carbon skeleton. *Acta Chem. Scand.* **18,** 77 (1964).
III. Ceder, O., Waisvisz, J. M., van der Hoeven, M. G. and Ryhage, R., Pimaricin 2: High pressure—high temperature hydrogenation studies. *Acta Chem. Scand.* **18,** 83 (1964).
IV. Ceder, O., Eriksson, G., Waisvisz, J. M. and van der Hoeven, M. G., Pimaricin 3: On the configuration of mycosamine. *Acta Chem. Scand.* **18,** 98 (1964).
V. Ceder, O., Pimaricin 4: Investigations on the pattern of unsaturation and oxygenation and the empirical formula. *Acta Chem. Scand.* **18,** 103 (1964).
VI. Ceder, O., Waisvisz, J. M., van der Hoeven, M. G. and Ryhage, R., Pimaricin 5: Determination of the size of the macrocyclic lactone ring and the position of mycosamine. *Acta Chem. Scand.* **18,** 111 (1964).
VII. Ceder, O., Pimaricin 6: Complete structure of the antibiotic. *Acta Chem. Scand.* **18,** 126 (1964).
VIII. Ceder, O. and Ryhage, R., The structure of filipin. *Acta Chem. Scand.* **18,** 558 (1964).

At the present time approximately fifty antibiotics believed to be polyene macrolides have been reported. So far complete structural determinations have been published for filipin, fungichromin, lagosin and pimaricin. Independent investigations using different methods have shown that fungichromin and lagosin possess identical gross structures.[16]

Fungichromin

Fungichromin was first isolated from cultures of *Streptomyces cellulosae* found in samples of soil collected in Greenville, Texas.[7] The pentaene nature was immediately recognized and the activity against yeasts and fungi was also determined.[7] The first report on the structure[8] dealt with the periodate oxidation of fungichromin and its dodecahydro derivative. The degradations, which are outlined in Fig. 1, describe the isolation of a fragment containing the pentaene chromophore. A summary of the reactions which finally led to the elucidation of the complete structure of the antibiotic is given in Fig. 2 (I). The key experiment was the conversion of fungichromin with lithium aluminum hydride and with hydroiodic acid—red phosphorus to 7,21-dimethyltritriacontane, 9. The structure of the hydrocarbon was proved by mass spectrometry and by synthesis. This finding established the carbon skeleton of fungichromin and allowed the empirical formula, $C_{35}H_{58}O_{12}$, to be calculated from the elemental analyses. The oxygenation pattern in fungichromin was determined by identification of the dibasic acids resulting from the oxidations of the antibiotic and of its dodecahydro derivative. The presence and position of the C_6-side chain was deduced from the isolation of hexanal from fungichromin treated with base and from the formation of caproic acid during the chromic acid oxidation of fungichromin and of dodecahydrofungichromin. The positions of the vicinal glycol systems were determined from investigations of the products of the periodate oxidation of fungichromin and of dodecahydrofungichromin.

The presence of only two atoms of deuterium in the diol, obtained when dodecahydrofungichromin was reduced with lithium aluminum deuteride, proved the absence of keto groups in fungichromin. This conclusion was verified by reduction of the C_{19}-fragment, obtained by periodate cleavage of fungichromin, with lithium aluminum deuteride and determination of the number

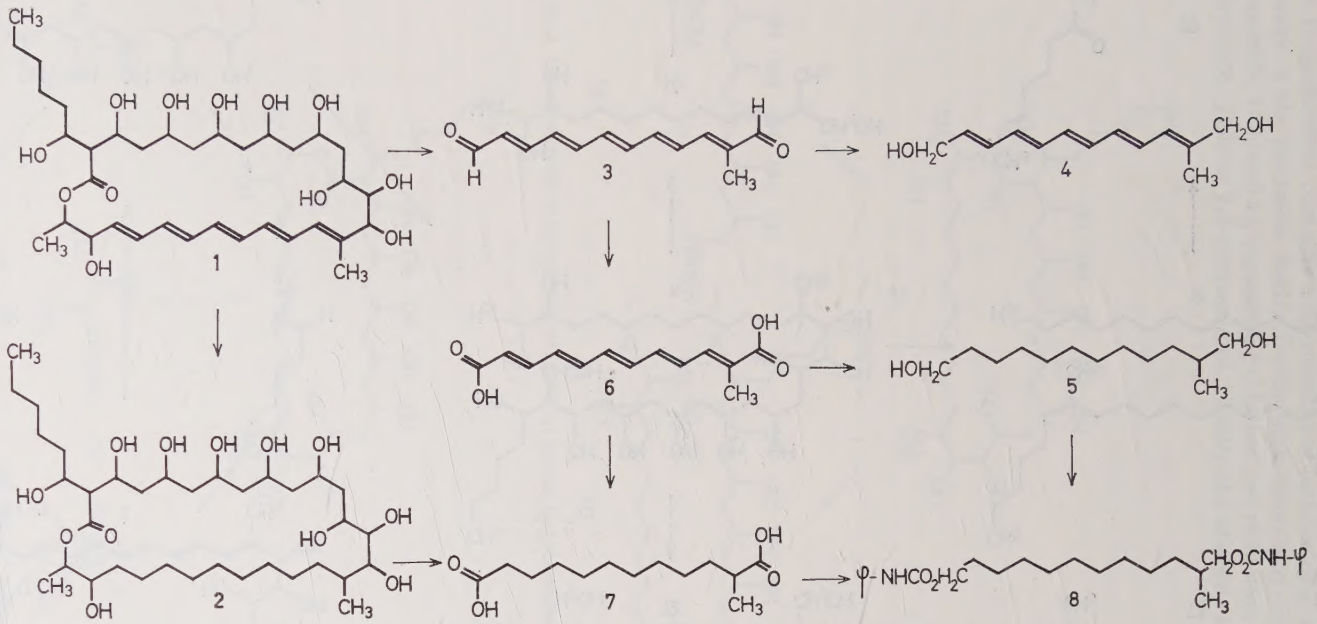


Fig. 1. Determination of the position of the pentaene chromophore in fungichromin.

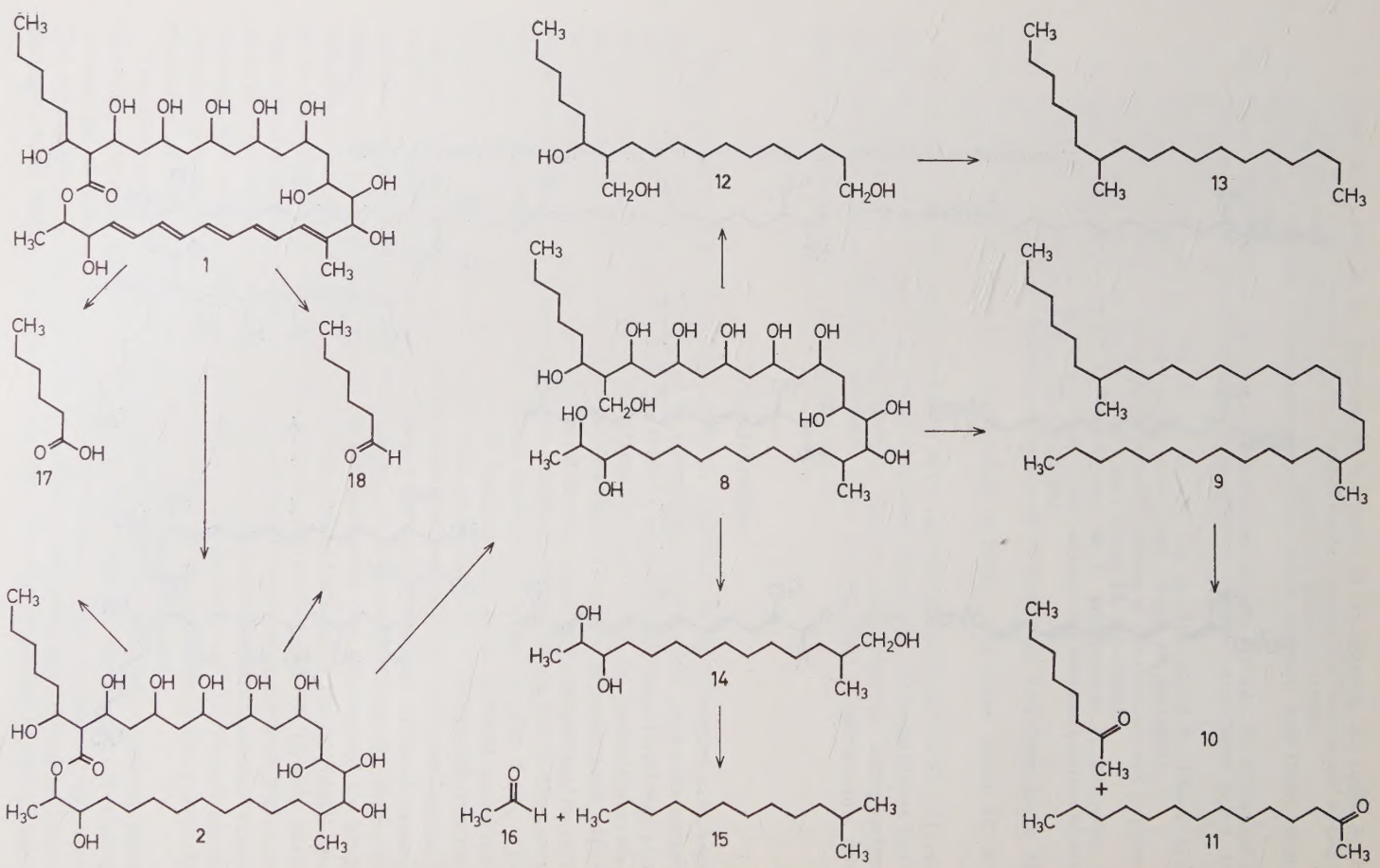


Fig. 2. Structural elucidation of fungichromin.

of deuterium atoms introduced in the resulting C_{19}-polyol, 19 (Fig. 3). It was proved that fungichromin contains a 28-membered lactone ring as outlined in Fig. 4. Cleavage of the pentaene double bonds with ozone and catalytic reduction of the ozonide, followed by periodate oxidation and reduction with lithium aluminum hydride, yielded 1,2-propanediol. Finally the absolute configuration

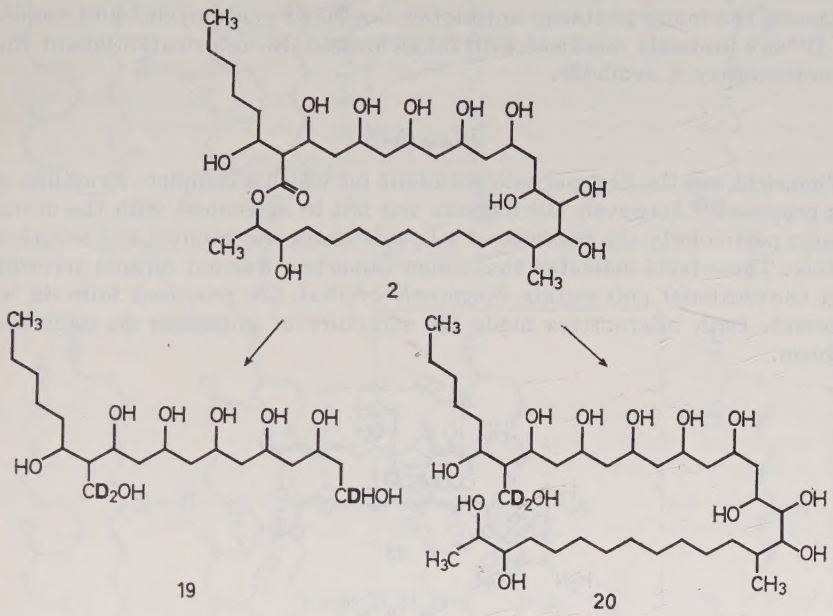


Fig. 3. Determination of the number of carbonyl groups in fungichromin.

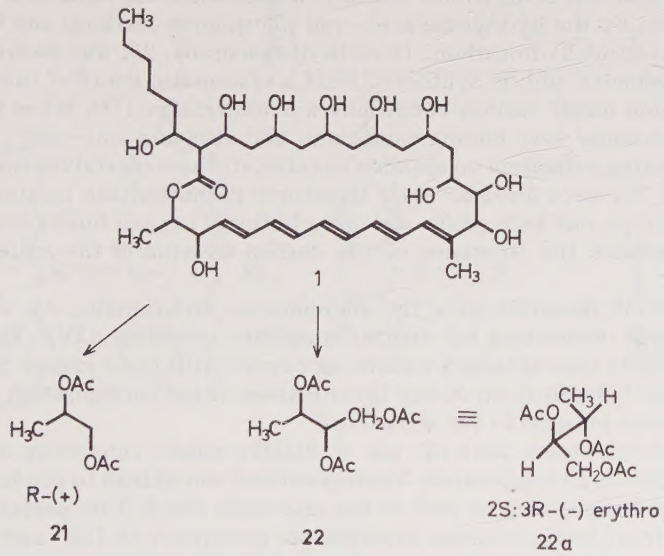


Fig. 4. Determination of the size of the macrocyclic lactone ring in fungichromin.

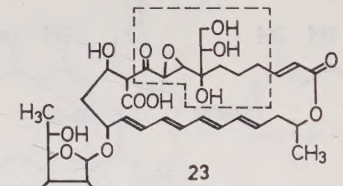
at C-26 and C-27 was derived from the isolation of *R*(+)-1,2-propanediol acetate, 21, and of 2*S*:3*R*(-)-*erythro*-1,2,3-butanetriol triacetate, 22, from the ozonide. The configurations were established by direct comparison with synthetic material.

It has later been reported[5a] that C-26 and C-27 in lagosin have an *erythro* configuration; thus fungichromin and lagosin are identical in this respect.

Among the many pentaene antibiotics described pentamycin[9] and moldicidin B[10] are probably identical with fungichromin. No information about their stereochemistry is available.

Pimaricin

Pimaricin was the first polyene antibiotic for which a complete structure, 23, was proposed.[11] However, the formula was not in agreement with the acetate theory; particularly the presence of a C_2-side chain was a novel and surprising feature. These facts indicated that either pimaricin was not formed according to a conventional polyacetate biogenesis or that the proposed formula was incorrect. Both alternatives made the structure of pimaricin an interesting problem.



23

$C_{34}H_{49}O_{14}N$

The first task was to determine the carbon skeleton of the antibiotic (II). This was achieved by the hydroiodic acid—red phosphorus method, and the structure of the parent hydrocarbon, 12-methylhexacosane, 25, was ascertained by mass spectrometry and by synthesis. Next a systematic study of the catalytic hydrogenation under various conditions was undertaken (II). When pimaricin was reduced under very forcing conditions, 200 atm and 250—300°, in glacial acetic acid using palladium on alumina as catalyst, three crystalline compounds, 27, 28, and 29, were formed. Their structures permitted the location of two carboxyl groups, one keto group and one additional oxygen function in pimaricin. Furthermore the structure of the carbon skeleton of the antibiotic was confirmed.

Acetolysis of pimaricin gave the aminohexose mycosamine, 34, whose configuration was determined by nuclear magnetic resonance (IV). The stereochemical results thus obtained were in agreement with those earlier arrived at by synthesis.[12] Recently an X-ray investigation of the configuration of mycosamine has been brought to our attention.[13]

It seemed reasonable that the use of slightly milder conditions during the high pressure—high temperature hydrogenations would lead to products which retained the lactone ring as well as the glycosidic bond. This postulation was confirmed from hydrogenation experiments performed at 150° and 150 atm, and compounds 35 and 36 were isolated (VI). Their structures imply that

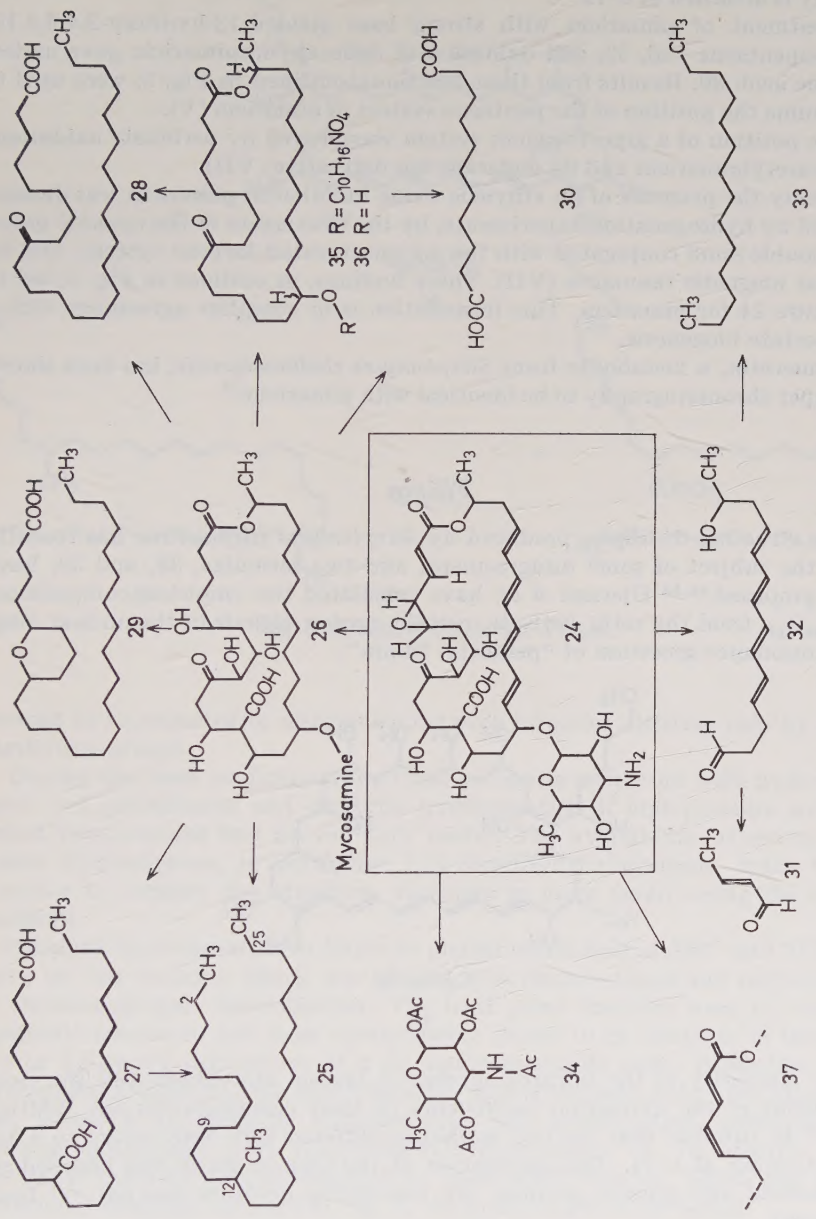


Fig. 5. Structural elucidation of pimaricin.

pimaricin contains a 27-membered ring lactone and that the mycosamine moiety is attached at C-12.

Treatment of pimaricin with strong base yielded 13-hydroxy-2,4,6,8,10-dodecapentaene-1-al, 32, and oxidation of dodecahydropimaricin gave undecandioic acid, 30. Results from these reactions, outlined in Fig. 5, were used to determine the position of the pentaene system in pimaricin (V).

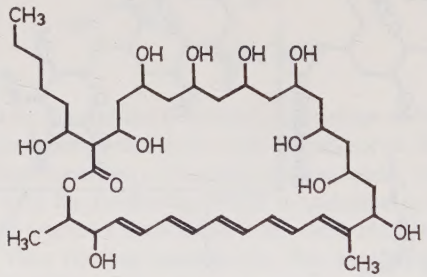
The position of a glycol-acyloin system was proved by periodate oxidations on *N*-acetylpimaricin and its dodecahydro derivative (VII).

Finally the presence of an ethylene oxide function in pimaricin was demonstrated by hydrogenation experiments, by the conversion of the epoxide group to a double bond conjugated with the α,β-unsaturated lactone system, and by nuclear magnetic resonance (VII). These findings, as outlined in Fig. 5, led to structure 24 for pimaricin. This formulation is in complete agreement with a polyacetate biogenesis.

Tennecetin, a metabolite from *Streptomyces chattanoogensis*, has been shown by paper chromatography to be identical with pimaricin.[14]

Filipin

The structure of filipin, produced by *Streptomyces filipinensis*, has recently been the subject of some disagreement, and two formulas, 38, and 39, have been proposed.[15,16] Djerassi *et al.* have calculated the empirical composition, $C_{37}H_{62}O_{12}$, from the ratio between certain proton signals in the nuclear magnetic resonance spectrum of "peracetyl filipin".



$C_{37}H_{62}O_{12}$

38

The similarity of the infrared spectra of lagosin and filipin and the close agreement of the extinction coefficients of their chromophores led Whiting *et al.*[16] to propose that the two antibiotics differed only with regard to a hydroxyl group at C-14. The assumption of the Oxford group was justified on biogenetical and general grounds, but convincing evidence has not yet been presented.

The "peracetyl filipin" used by the Stanford group was not obtained in a crystalline state. Experience with fungichromin and pimaricin had demonstrated the difficulty to eliminate the last traces of solvent even from crystalline derivatives (cf. I and V); therefore the application of the nuclear magnetic resonance method on a compound containing approximately 60 hydrogen atoms

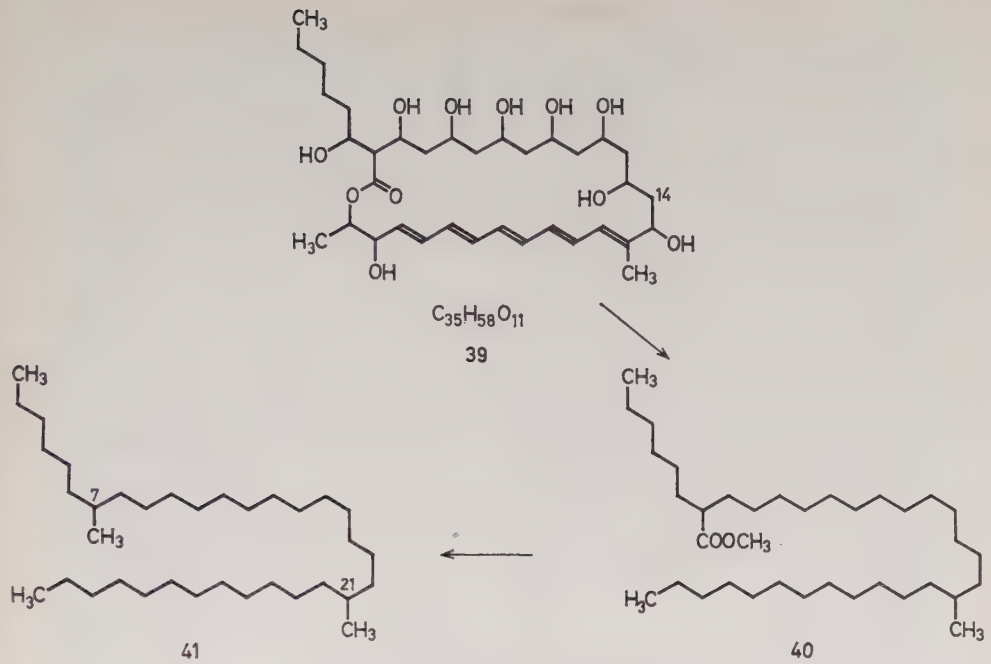


Fig. 6. Structural elucidation of filipin.

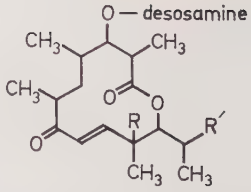
seemed to be unsuited to distinguish between formulas differing only by a few methylene groups.

During the work on fungichromin and pimaricin reduction with hydroiodic acid—red phosphorus and catalytic hydrogenation at high pressure and elevated temperatures had proved very useful. The availability of several synthetic hydrocarbons, in particular 7,21-dimethyltritriacontane, made it attractive to explore the structure of filipin in more detail, using the above methods.

Catalytic hydrogenation of filipin in glacial acetic acid at 280° and 212 atm gave an oily mixture which was treated with diazomethane and subjected to a chromatographic investigation. The least polar fractions were by nuclear magnetic resonance and mass spectrometry shown to be mixtures of isomeric 1,4 or 1,5-epoxy derivatives of a C_{35} monocarboxylic ester. Reduction with lithium aluminum hydride and treatment with hydroiodic acid—red phosphorus, followed by catalytic hydrogenation and esterification, produced gas chromatographically pure 7,21-dimethyltritriacontane, 41. Its identity with authentic material was ascertained by mass spectrometry. These and some additional findings, outlined in Fig. 6, led to structure 39 for filipin.

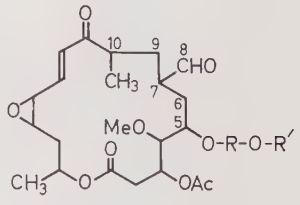
General aspects

The first complete structure of a macrolide, methymycin,[17] 42, was reported in 1956. It was in rapid succession followed by formulas for a large number of related metabolites: neomethymycin,[18] 43; erythromycin,[19] 44; erythromycin



42: R = OH R´ = H
43: R = H R´ = OH

44: R = OH R´ = cladinose
45: R = H R´ = -"-
46: R = OH R´ = $C_7H_{13}O_3$



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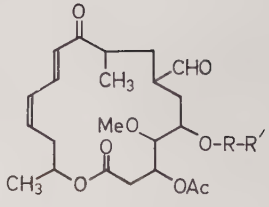

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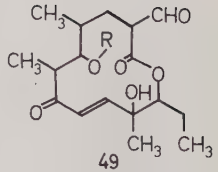
47 and 48 { R = mycaminose
R´ = isovalerylmycarose



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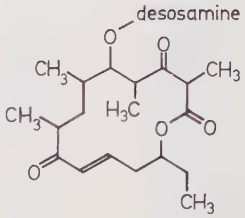
R = desosamine



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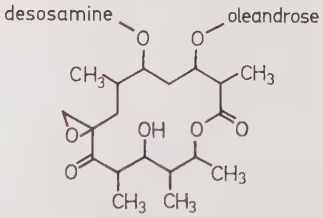

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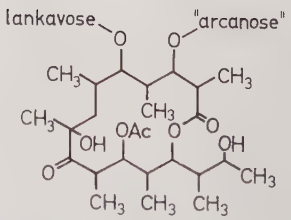

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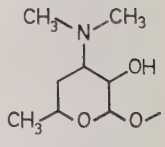


53

B,[20] 45; erythromycin C,[21] 46; magnamycin,[2] 47; magnamycin B,[2] 48; picromycin,[22, 23] 49; narbomycin,[24] 50; oleandomycin,[25] 51 and lankamycin,[26] 52.

The approach to determination of structure has generally been to degrade the aglycone part into relatively small fragments, that were used to deduce the complete formula of the antibiotic. Often the empirical composition could not be ascertained until the complete structure was known. The situation was well described by Gerzon *et al.*[27] who emphasized that "these transformation products *collectively rather than individually* furnish proof for" the structures of certain degradation products of erythromycin, and as a consequence also for the antibiotic itself. As larger and more complex macrolides are being studied, this method has become increasingly laborious. The situation has been partly improved by the application of reductive methods that yield products retaining all carbon atoms in the macrocyclic ring. The structures of these degradation products, free from hydroxyl groups, can be easily elucidated by physical methods and often confirmed by synthesis. This type of approach has considerably decreased the time required for a structural determination of macrolides.

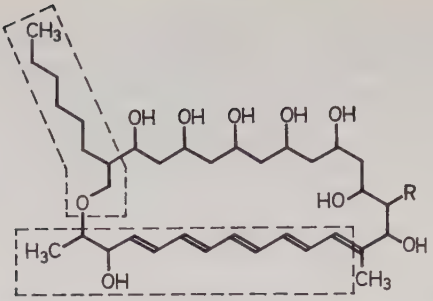
Since very few of the optically active degradation products from macrolides have been directly correlated with compounds of known configuration, none of these antibiotics has as yet been assigned absolute or relative configurations at all centers. To entirely solve these sterical problems by chemical methods would be very difficult and time consuming; X-ray diffraction has played a minor role in the macrolide group, and in fact no single crystallographic investigation has as yet been reported for any of its members (cf. however Ref. [16, 28]). Likely reasons are difficulties encountered in the preparation of suitable crystalline derivatives and in obtaining solvent-free samples. These obstacles are not easily overcome, but the less complicated problem of the conformation of the macrocyclic ring seems accessible at the present time.

Biogenesis

Since the first macrolide structures were reported, their biogenesis has attracted considerable interest. Tracer studies performed with erythromycin, 44, [29, 30] have shown that its lactone moiety is formed by the condensation of seven propionate units. Similar studies proved that methymycin, 42, was built up from five propionate and one acetate unit.[31] Magnamycin, 47, however, displays a slightly unexpected pattern. It has been shown by tracer studies that C-5, C-6, C-7, and C-8, which include the aldehyde group, are derived from glucose in the fermentation medium.[32] By the same method, Grisebach and Achenbach also demonstrated that acetate was not incorporated into the aldehyde group, C-5, C-6 or C-7, and that C-9, C-10 and the methyl group on C-10 originated from propionic or methylmalonic acid.[33] It is also of interest to note that methymycin, labelled almost entirely in the *N*-methyl carbons of the desosamine moiety, 53, resulted when the fermentation was carried out in the presence of [*methyl*-^{14}C]-methionine. No *C*-methylation could be detected.[31]

Only one biosynthetic isotope experiment has been reported for the polyene macrolides. It was demonstrated that labelled acetate, but not malonate, was incorporated into the aglycone of nystatin.[34, 35] This finding incidentally led to the proposal that the radioactivity of the sample can be used to estimate the number of carbon atoms in the aglycone.

The important observation has been made that maximum yield of fungichromin is obtained only when the culture medium contains optimal amounts



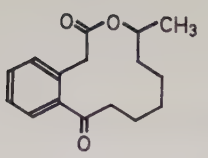
54

of oleic acid. It therefore seems conceivable that à substantial part of the carbon skeleton, most likely the carbon atoms of the polyene system, are derived from oleic acid. It is not known, however, whether a metabolite of oleic acid, such as a C_2-unit, is used. Likewise the role of the carbohydrates in the biosynthesis of the polyene macrolides is incompletely understood. The parts of the common carbon skeleton of filipin, fungichromin and lagosin, which are most likely to have originated from a fatty acid, are indicated in structure 54.

The pentaenes and the hexaenes are the only macrolides known, which lack the sugar moiety. The fact that sugar-free saturated macrolides have never been isolated could possibly be due to the absence of easily detectable properties, like color or biological activity. Metabolites with identical carbon skeletons but differing in respect to oxidation state or oxygenation pattern are well-known, e.g., methymycin—neomethymycin—picromycin, magnamycin—magnamycin A, and erythromycin—erythromycin B. As yet, however, homologues with carbon skeletons differing by one or a few carbon atoms have never been isolated from the same microorganism. This circumstance may only reflect the shortcoming of our separation methods, but it is very likely the result of a high degree of specificity of the enzymes involved in their synthesis. It is notable that all structurally investigated macrolides, except neomethymycin[18] and lankamycin,[26] have the macrocyclic lactone ring closed at the last hydroxyl group of the fatty acid chain. A comparison of the absolute configuration of the corresponding asymmetric carbon atom in different macrolides would be of considerable interest.

Filipin, fungichromin and lagosin form a congeneric group which may provide valuable biogenetic information. The small difference in specific rotation between the structurally identical pair fungichromin—lagosin and the lack of a hydroxyl group at C-14 in filipin could indicate that one of the final steps in the biosynthesis of these polyenes consists of a reaction between a C_{16}- and a C_{19}-fragment, synthesized separately. If it could be demonstrated that fungichromin and lagosin possessed opposite configurations at C-14, such a theory would gain in probability.* A possible consequence of such a mechanism could be that the closure of the macrocyclic lactone ring is not a late or final step in the biosynthesis of macrolides. If the first reaction were the formation of an ester, it would be easily explained that the last hydroxyl group of the fatty acid chain

* This interrelation was proposed by Dr. M. C. Whiting during a discussion on Memorial Drive, Cambridge, Mass. on July 8, 1961.



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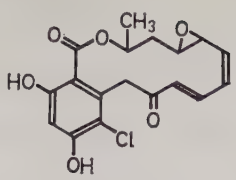

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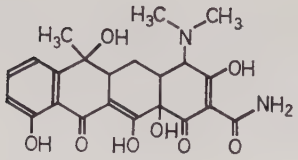

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is lactonized in most macrolides, regardless of the ring size. The two carbon chains of the ester could be extended by the same or by different biogenetic processes. The formation of a carbon-carbon bond yielding the macrocyclic ring would then be the final biosynthetic step. The part of magnamycin derived from glucose (C-5 to C-8) is surrounded by carbon atoms of acetate origin, a fact that is not in conflict with an "ester hypothesis".

The structures of curvularin,[37] 55, and radicicol,[38] 56, indicate that the macrolide antibiotics could be precursors to the aromatic or partially aromatic metabolites of the tetracycline type,[39, 40] 57, which are also produced by *Streptomyces* strains. The tetracyclines have been shown to have an acetate origin,[41] and it is easy to conceive that they could be formed by transannular condensations in a macrolide.

The work on pimaricin and filipin was carried out at the Royal Institute of Technology. I am much indebted to Professor H. Erdtman for his kind interest and support.

I wish to thank Professor A. C. Cope of the Massachusetts Institute of Technology for the pleasant and fruitful time I spent in his laboratory.

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